Estimated prevalence of Johne's disease in herds of water buffaloes

**B(ubalus bubalis)** in the province of Caserta

Gaetano Desio,1 Sandra Nizza,1 Serena Montagnaro,1 Simona Sassò,1 Luisa De Martino,1 Valentina Iovane,1 Roberto Ciarcia,4 Francesco Casalnuovo,3 Ugo Pagnini2

1Dipartimento di Patologia e Sanità Animale, Università Federico II di Napoli, Italy
2Dipartimento di Strutture, Funzioni e Tecnologie Biologiche, Università Federico II di Napoli, Italy
3Istituto Zooprofiliattico Sperimentale del Mezzogiorno, Sezione diagnostica provinciale di Catanzaro, Italy

**Abstract**

Paratuberculosis is a chronic infection of domestic and wild ruminants, caused by Mycobacterium avium subsp. paratuberculosis. The persistence of paratuberculosis infection for months up to years without exhibiting any clinical signs makes the diagnosis and control program a difficult proposition. Limited informations on prevalence of paratuberculosis in water buffaloes (Bubalus bubalis) are available. We carried out a study on 1350 buffaloes belonging to 56 herds in the Caserta province, of Campania region, Italy. The prevalence of infected buffalo dairy herds was estimated by a commercial ELISA kit of individual blood samples of animals over 24 months of age. On the basis of performance (sensitivity 43%, specificity 99.3%) of ELISA test on serum, the resulting true prevalence at animal level and at herd level was 4% (95% CI 3% to 5%) and 74.1% (95% CI 71.8% to 76%). Considering the paucity of epidemiological reports in the region our results could be a useful contribution towards the prevention of buffalo paratuberculosis in the area.

Corresponding author: Dr. Serena Montagnaro, Università Federico II di Napoli, Dipartimento di Patologia e Sanità Animale, Scuola di Medicina Veterinaria, via Delpino 1, 80137 Napoli, Italy.
Tel.: +39.081.2536178 - Fax: +39.081.2536179.
E-mail: serena.montagnaro@unina.it

Key words: Paratuberculosis, Water Buffalo, ELISA, Seroprevalence.
Acknowledgments: the authors were supported by grant No. IZS ME 12/07 RC.
The authors gratefully acknowledge the careful reading of the manuscript by Dr. F. Di Pascale and the technical contribution of Dr. E. Manco and Dr. G. Visone.

Received for publication: 7 June 2012.
Last revision received: 1 October 2012.
Accepted for publication: 13 November 2012.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright G. Desio et al., 2013
Licensee PAGEPress, Italy
Italian Journal of Animal Science 2013; 11:e8
doi: 10.4081/ijas.2013.e8

**Introduction**

*Mycobacterium avium* subsp. *paratuberculosis* is the causative agent of the chronic disease paratuberculosis (also known as Johne's disease) of cattle and other ruminants (Chiodini et al., 1984). The incubation period has a variable duration, but in general ruminants do not develop clinical signs of the disease before 2 years of age (Whitlock and Buergeit, 1996). The transmission of infection to uninfected cattle usually occurs early in life; cattle up to 1 year of age considered to be most susceptible. Infection generally occurs through contaminated feed, water, milk and colostrum (Sweeney et al., 2000). Clinical signs are usually observed in older cattle and include diarrhea, decline in milk production, severe weight loss despite good appetite and ultimately death. However, most infected cattle are never clinically affected (Woodbine et al., 2009).

Johne's disease (JD) is linked to significant economic losses, in fact, in United States, costs due to reduced productivity have been estimated high in herds where the prevalence of clinical cases among culled animals was >10% (Ott et al., 1999), owing to reduced milk production, premature culling, and reduced cull value (Wells and Wagner, 2000). Because of its impact on herd productivity (along with the potential public health consequences related to the fact that *Mycobacterium avium* subsp. *paratuberculosis* has been linked to Crohn's disease in humans) (Mishina et al., 1996; Hermann-Taylor et al., 2000), understanding JD and how is the best way to manage and control its spread within and between herds is gaining interest from the livestock industry (Linnabary et al., 2001).

In addition to these production losses, infection with *M. paratuberculosis* is causing problems with the National Tuberculosis Control Programme by sensitizing buffaloes and cows to mycobacterial antigens so they could react to the Tubercolin test for *Mycobacterium tuberculosis* var. *bovis* (OIE Manual 2009). Many factors can influence the prevalence and spread of paratuberculosis. Several individual and management practices have been identified as potential risk factors for the introduction and spread of the disease (Pillars et al., 2011; Nielsen et al., 2003). There are a number of studies available which evaluate the potential risk factors associated with the transmission of paratuberculosis in cattle (Collins et al., 1994; Johnson-Feareruluundu and Kaneene, 1999; Daniels et al., 2002; Raizman et al., 2005). However, less is known about the transmission and spread of *Mycobacterium avium* subsp. *paratuberculosis* in buffalo.

For water buffaloes, bred in Caserta province, little is known about the spread of paratuberculosis. Studies are usually carried out almost only on bovine (*Bos taurus*), which is genetically different from buffalo species (*Bubalus bubalis*) present in our region and is also characterized by another target production (Montagnaro et al., 2008).

Breeding of water buffaloes is an economically important zootechnic reality in our country, especially in Caserta province (Campania region, south of Italy) due to the international level of its DOP products (buffalo's mozzarella cheese).
There have been discussions in Europe regarding Johne’s disease control program on a region-by-region basis or even at national level (EFSA, 2004) for establishing the effectiveness of control programmes, baseline prevalence data are needed, especially for different geographical areas and management systems. The recommendations set out in management/control programmes are generally complex, and it is difficult to determine which are the key elements for Johne’s disease control (Ridge et al., 2005; Cenci-Goga et al., 2010).

Hence, a more comprehensive knowledge of Mycobacterium avium subsp. paratuberculosis seroprevalence in buffaloes is of great value to facilitate the design of prevention and control programmes. Understanding the transmission dynamics of Mycobacterium avium subsp. paratuberculosis is a key element of control programmes that aim to reduce, or preferably eliminate, it from buffalo dairy farms. The objective of the current study, therefore, is to describe, by a cross-sectional study, the seroprevalence and the relationship between buffalo farm management and transmission of Mycobacterium avium subsp. paratuberculosis in buffalo dairy herds of Campania region, Caserta province, in which the production of buffalo’s milk and mozzarella cheese are mainly concentrated.

Materials and methods

Study design

The study was based on two conditions: the selection of a geographic environment where the production of buffalo’s mozzarella cheese is mainly concentrated and the farms selection that raise dairy buffalo only.

A cross-sectional study was carried out from January 2010 to January 2011. Sample size was calculated based on the list of dairy farms registered in Caserta province. Using an expected true herd prevalence of 8.8% (Cenci-Goga et al., 2010) and a confidence interval of 99% meaning that a total of 1350 buffaloes from 56 dairy farms were randomly selected using the program WinEpiscope 2.0®. All tested herds had at least 40 buffaloes, to simplify the sample collection process, the number of samples per herd was fixed at 25, only buffaloes of at least 2 years old were sampled.

General information about individual characteristics, farm management practices, farm characteristics, animal health and veterinary services were obtained via a questionnaire that was administered to the managers of the farms.

Laboratory analysis

Blood was collected by tail vein venepuncture into anticoagulant free vacutainer tubes. Serum was separated by centrifugation and aspiration and stored at -70°C until analysis by enzyme linked immuno assay (ELISA).

A commercial ELISA kit (ID SCREEN®, Paratuberculosis Indirect ELISA kit) for detection of antibodies against Mycobacterium avium subsp. paratuberculosis in buffalo serum samples was used, the kit has been supplied from IDVet (Innovative Diagnostics, France). The principle of the test depends on indirect ELISA, Sera were tested according to the manufacturer’s instructions, the absorbance reading OD in all ELISA plate wells were measured at 450 nm using an automated ELISA reader. ELISA optical density (OD) readings were transformed to Serum/Positive percentage (S/P%) according to a specific equation cited by manufacturer. The sample was considered positive if it gave S/P% ≥ 70%, doubtful if 60% < S/P% < 70%, negative if S/P% ≤ 60%. S/P% = (OD sample-ODNC)/(ODPC-ODNC).

The negative and positive control samples provided by the manufacturer were run in duplicate on each plate.

Data analysis

The apparent prevalence of Mycobacterium avium subsp. paratuberculosis infection at individual animal level was calculated as the number of serologically positive buffaloes among the total number of buffaloes tested. At individual animal level, a positive serological result was defined in term of cut-off of the ELISA test.

The apparent prevalence of Mycobacterium avium subsp. paratuberculosis infection at herd level was calculated as the number of serologically positive herds among the total number of herds tested. Using the manufacturer’s suggested cut-off point, an individual herd is classified as positive on the basis of having one or more seropositive animals. The true prevalence TP was estimated, at animal level and at herd level, using standard methods (Thursfield, 1995):

\[
TP = \frac{(AP + Sp - 1)}{(Se + Sp - 1)}
\]

where:

- AP is the apparent prevalence;
- Se is the sensitivity of the test;
- Sp is the specificity of the test.

Univariate analysis was used to estimate differences in distribution of management factors between seronegative and seropositive herds. Variables that had a level of significance (P-value) of <0.30 in univariable analysis were included for multivariate analysis. A backward elimination procedure was used to estimate the relationship between serological status of the herd and management factors, using a generalized linear model (SAS, 1989). The response (seropositive or seronegative) was modelled by using a binominal probability distribution and the fixed and random components of the model were linked with a logit-link function.

Results

A sample composed of 56 herds was taken from the 560 dairy buffalo herds in the Caserta province. All of the 56 randomly selected herds received a questionnaire which was completed and the cattle were tested serologically. From this sample, 1350 animals were tested in duplicate for antibodies against Mycobacterium avium subsp. paratuberculosis by ELISA. Of the 1350 buffaloes tested, using a sensitivity of 45% and specificity of 99.3%, as claimed by the manufacturer, a total of 37 buffalo serum samples were found to be serologically positive to Mycobacterium avium subsp. paratuberculosis and the total apparent prevalence was 2.7% (95% CI 1.9% to 3.5%). However, when the apparent prevalence was adjusted for the relative sensitivity and specificity of the ELISA, using the standard method, the expected true prevalence was 4% (95% CI 3% to 5%).

Using the manufacturer’s suggested cut-off point, herd prevalence was also estimated. Of the 56 herds tested, 18 were found to have at least one positive buffalo that gives an apparent herd prevalence of 32.14% (95% CI 20% to 44.2%). The standard method was used to derive the herd true prevalence from herd apparent prevalence, and the estimate true prevalence at herd level was 74.1% (95% CI 71.8% to 76.4%) (Table 1).

Table 1. Apparent and true prevalence, with 95% confidence intervals (CI), of Mycobacterium avium subsp. Paratuberculosis in buffaloes dairy herds of Caserta Province.

<table>
<thead>
<tr>
<th>Level</th>
<th>Apparent prevalence</th>
<th>True prevalence</th>
<th>Number of positive</th>
<th>Number of negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>2.7% (95% CI 1.9% to 3.5%)</td>
<td>4% (95% CI 3% to 5%)</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>Herds level</td>
<td>32.14% (95% CI 20% to 44.2%)</td>
<td>74.1% (95% CI 71.8% to 76.4%)</td>
<td>37</td>
<td>1313</td>
</tr>
</tbody>
</table>
Discussion

*Mycobacterium paratuberculosis* has been detected in dairy buffaloes herds throughout Italy (Lillini et al., 1999, 2002, 2005; Robbi et al., 2002; Arrigoni et al., 2007; Cenci-Goga et al., 2010). Our study has confirmed that *M. paratuberculosis* is also present in buffalo dairy herds in Caserta Province, in Campania Region. By ELISA, the apparent animal level prevalence for Caserta Province was 2.7%±0.8%. These levels of infection are higher than those reported by Lillini et al. (2002) (0.21%) and that reported by Sezzi et al. (2010) (0.22%) for buffalo dairy herd in Lazio Region using ELISA technique. Similar levels of infection have been reported, in cattle, in Lazio Region (2.4%) (Lillini et al., 2005) and Veneto Region (3.5%) (Robbi et al., 2002) but the seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* found in the present study is lower than the AP previously reported, in cattle, for other Italian region and for other part of the EU and USA (Cenci-Goga et al., 2008; Arrigoni et al., 2007; Nielsen et al., 2000; Pillars et al., 2009; Pence et al., 2003). Such differences could be due to different causes, in fact the accuracy and precision of prevalence estimates are influenced by the sampling methods, diagnostic tests employed and the variability of the condition within the population being examined (NcNab et al., 1991). Thus, the prevalence value could be influenced by use of less sensitive assays. For example, in a pilot study in India using an indigenous and in-house plate ELISA kit (with protoplasmatic antigen from native *Mycobacterium avium* sbsp. *paratuberculosis* Bison type strain) the seroprevalence was 23.3% in buffaloes (12.1% in young and 24.4% in adults) (Singh et al., 2008). Also the target population can modify the prevalence value, in fact, for *Mycobacterium avium* subsp. *paratuberculosis* infections, ELISA will usually not detect infected animals less than 2 years of age, as these have not developed antibodies to *Mycobacterium avium* subsp. *paratuberculosis* (Nielsen and Erssbl, 2006). Moreover reduction of bias caused by a diagnostic test can be reduced by inclusion of age-specific test accuracy estimates (Sergeant et al., 2008). Therefore, it is difficult to compare prevalence estimates derived from different populations using different sampling methods and tests (NcNab et al., 1991).

The results between the two groups of herds, infected and not infected, were significantly different only concerning the presence of calving area and suckling calves. A calving area was present on 100% and 33% of seronegative and seropositive herds respectively (P<0.01). The percentage of herds feeding only milk replacers was higher (83%) in the seronegative herds than in the seropositive herds (67%) (P<0.01). In the multivariable analysis, the presence of calving area and feeding only milk replacers were the only risk factors associated with serological status: herds without calving area and with suckling calves were associated (P<0.01) with increased risk of a seropositive status.

Analysis of risk factors associated with a herd being positive for *Mycobacterium avium* subsp. *paratuberculosis* was within the scope of this study. On nearly 80% of seropositive farms calves were not born in a cleaned calving area and the farmers failed to separate the calves from their mothers immediately after birth. This still indicates that *paratuberculosis* calving management is an important risk factor (Johnson-Ifearulundu and Kaneene, 1998; Wraight et al., 2008). Therefore, it is difficult to compare prevalence estimates derived from different populations using different sampling methods and tests (NcNab et al., 1991).

A large number of farms (83%) 0-6 months old calves housed in a separate building away from older buffaloes and calves were fed only milk replacers, a management practice that decreases the possibility of transmitting contaminated faeces directly from adults to buffalo calves. The ingestion of *Mycobacterium avium* subsp. *paratuberculosis* by colostrum, milk and forage is generally considered a risk. After birth, colostrum can provide the immunoglobulins calves require to develop an efficient passive immunity, and for this, colostrum substitutes are less effective (Garry et al., 1996). The disadvantage is that the colostrum may be contaminated with *Mycobacterium avium* subsp. *paratuberculosis* (Streeter et al., 1995); feeding the colostrum of the calf’s own mother instead of feed pooled colostrum reduces the risk of infection. However, a minority of farmers continue to feed pooled colostrum. *Paratuberculosis* is widespread in ruminant populations in almost all countries with a dairy industry. In several countries, disease-control programs are developed to reduce *Mycobacterium avium* subsp. *paratuberculosis* prevalence in the participating dairy farms (Shin, 1989; Benedictus et al., 2000; Wells et al., 2002). Currently, there is no nationwide programme to control Johnne’s disease in Italy, nor is there a regional programme in Campania, so our data provide a general indication of a true state of *Mycobacterium avium* subsp. *paratuberculosis* infection in buffalo in Caserta Province, and suggest that a control program could be considered to control the disease.

To control the spread of *Mycobacterium avium* subsp. *paratuberculosis*, test-and-cull interventions are typically recommended (Stott et al., 2005; Tiwari et al., 2005; Lu et al., 2008) but a single test-and-cull strategy may be inadequate because the available tests have low sensitivity (Whitlock et al., 2000), and literature data (Sezzi et al., 2010; Lillini et al., 2002) have shown that ELISA tests currently available for *Mycobacterium avium* subsp. *paratuberculosis* diagnosis for bovine animals could be inadequate for use in water buffaloes. Thus, repeat testing and culling could be more effective because the sensitivity of the test is increased with repeated usage; however, it has to be taken into account that the diagnosis of Johnne’s disease is a major burden for the farming industry, and these test-and-cull strategies without closing infection routes are ineffective in reducing the prevalence and are not cost-effective (Kudahl et al., 2008; Cenci-Goga et al., 2010).

Besides transmitting the infection to other ruminants, infected buffaloes may act as a reservoir of infection for other species, such as humans. In fact, *Mycobacterium avium* subsp. *paratuberculosis* has been linked to human Crohn’s disease (Mishina et al., 1996; Hermon-Taylor et al., 2000; Sezzi et al., 2010); the way of transmission of *Mycobacterium avium* subsp. *paratuberculosis* is not yet fully understood but some previous evidence focuses on food products as the transmission pathway (Cocito et al., 2001). *M. paratuberculosis* has been recovered after simulated high-temperature, short-time pasteurization of naturally infected milk, has been cultured from pasteurized milk for retail sale (Grant et al., 1996; 2002a; 2002b) and was detected in Swiss hard and semihard cheese (Spahr et al., 2001) and Retail Cheeses from Greece and the Czech Republic (Ikonomopoulos et al., 2005). The main produce of Italian buffalo breeding are milk and cheese (such DOP product: mozzarella di bufala campana). Therefore, the possibility exists that the milk for mozzarella production may contain viable *M. paratuberculosis* cells.

Conclusions

For all these reasons the economic importance of this animal species, linked to trading of high quality milk and cheese, requires a correct dairy management and a sanitary control programme with the purpose of eradicating this and/or other infectious diseases especially when viable mycobacteria could be present in cheese and represent a potential risk to human health (Grant et al., 2001).

References


http://www.aspajournal.it/index.php/ijas/rt/printerFriendly/ijas.2013.6#2275
23. Lillini, E., Gamberale, F., Di Guardo, G., 1999. Mycobacterium avium subsp. paratuberculosis infection in a water-buffalo (Bubalus bubalis) from central Italy. Page 254 in Proc. 6th Int. Colloquium on Paratuberculosis, Madison, WI, USA.